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NTP Research Concept - Alkylaniline

Scott S. Auerbach Ph.D.
Study Scientist
National Institute of Environmental Health Sciences

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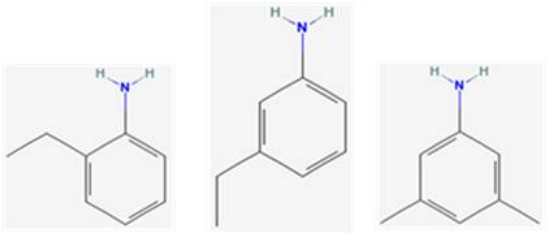
Nomination



- 2-ethylaniline (2-EA), 3-ethylaniline (3-EA), and 3,5-dimethylaniline (3,5-DMA) were nominated by NIEHS for toxicological characterization
- Justification
  - ubiquitous potential exposure scenarios
  - limited availability of published toxicological data for this subclass of alkyl-substituted anilines
  - structural similarities to the known animal carcinogens 2,6-xylidine (2,6-DMA), and *o*-toluidine (2-MA)

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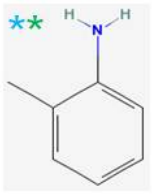
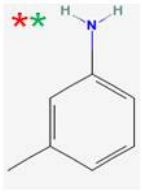
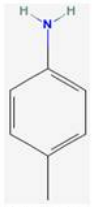
Monomethyl substituted



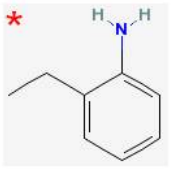
2-methylaniline



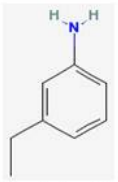
3-methylaniline


4-methylaniline

Monoethyl substituted

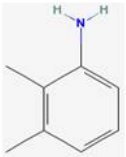


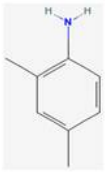
2-ethylaniline

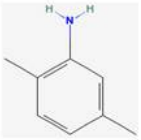

3-ethylaniline

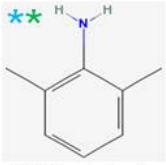

4-ethylaniline

Dimethyl substituted

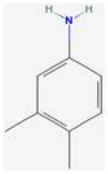

2,3-dimethylaniline

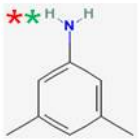

2,4-dimethylaniline


2,5-dimethylaniline



2,6-dimethylaniline

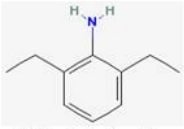

3,4-dimethylaniline

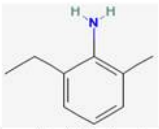


3,5-dimethylaniline

* Nominated chemical
* Rodent carcinogen
* Associated with human bladder cancer

Others with HPV status


2,6-diethylaniline


2-methyl-6-ethylaniline

Uses

Reactants and chemical intermediates in the production of:

- Antioxidants
- Antiozanants
- Azo dyes
- Curing agents
- Detergents
- Gasoline additives
- Pesticides
- Pharmaceuticals
- Special lacquers
- Wood preservatives

Human exposure

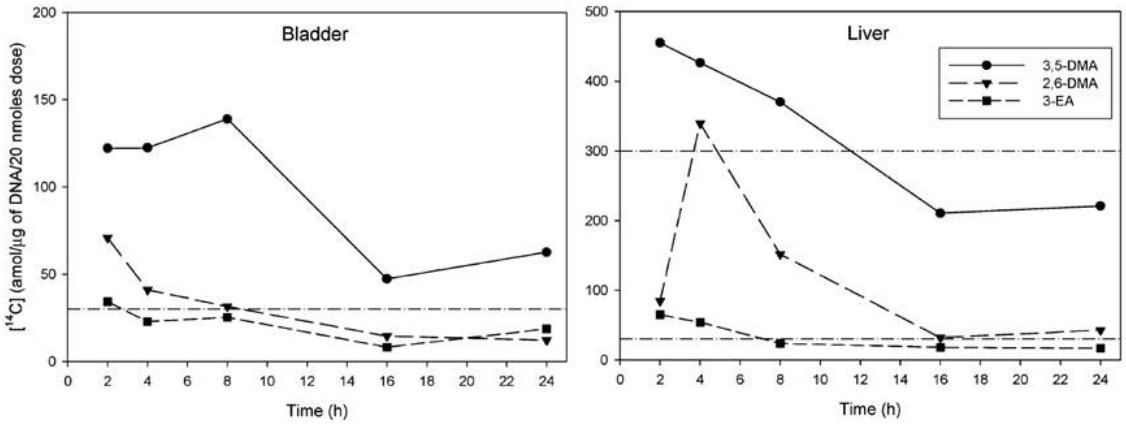
- Biomonitoring studies (Hemoglobin (Hb) adducts) have indicated widespread human exposure to a variety of alkylanilines
  - Hb adduct levels of certain alkylanilines were associated with bladder cancer
- Likely primary source of widespread exposure
  - mainstream and sidestream cigarette smoke
- Occupational exposure
  - Possible inhalation and dermal exposure during production and use
- Other potential sources
  - drugs
    - metabolism of lidocaine
  - pesticides
    - biodegradation/metabolism of chloroacetanilide herbicides and phenylamide fungicides
  - food
    - metabolism of xylazine

Background

- Toxicity: methemoglobinemia; irritant
- Carcinogenicity of structurally related alkylanilines
  - 2-MA (NTP study)
    - Mouse: liver
    - Rat: Mammary gland, subcutaneous tissue and bladder
    - IARC: Group 2A = probably carcinogenic to humans
  - 2,6-DMA (NTP study)
    - Mouse: no study
    - Rat: Nasal cavity, subcutaneous tissue and liver
    - IARC: Group 2B = possibly carcinogenic to humans

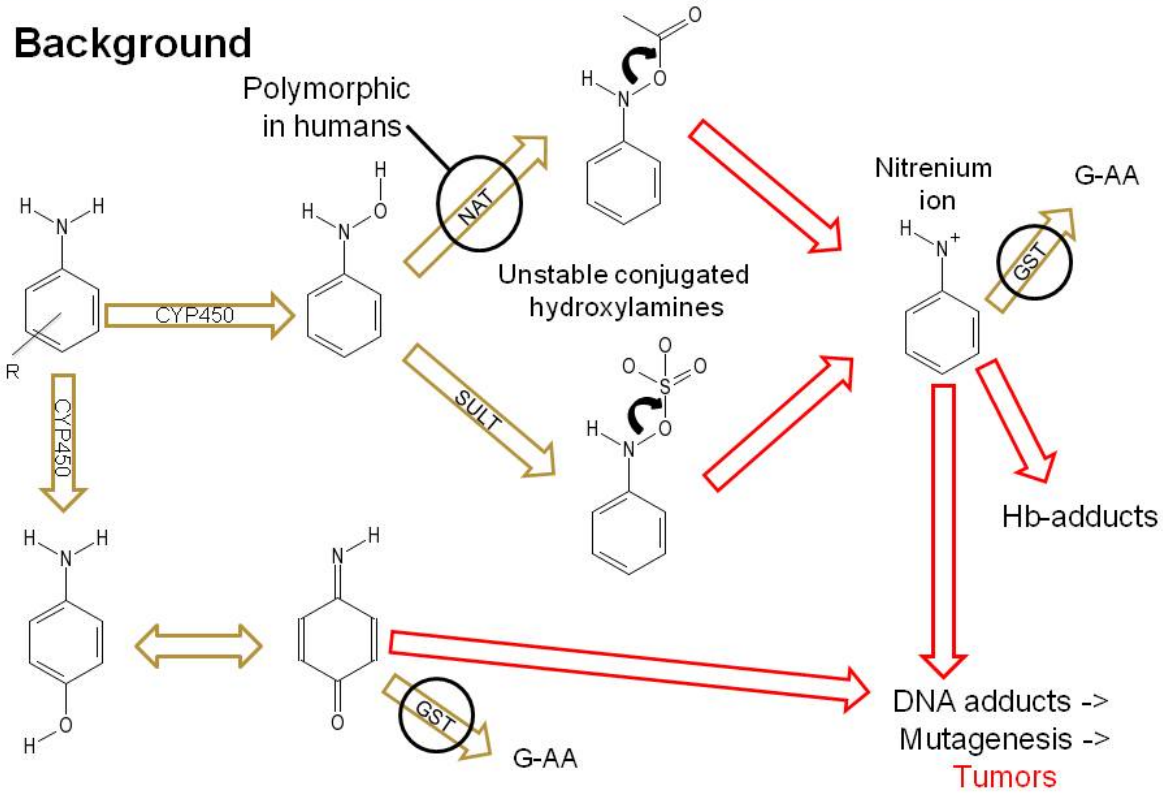
Background

- Genotoxicity of the alkylanilines
  - Generally negative in Ames mutagenicity studies
  - Generally positive for clastogenicity
  - Some form DNA adducts *in vivo*



Skipper, et al., CRT, 2006

Background


Polymorphic
in humans
NAT
CYP450
CYP450
SULT
Unstable conjugated
hydroxylamines
Nitrenium
ion
G-AA
GST
Hb-adducts
GST
G-AA
DNA adducts ->
Mutagenesis ->
Tumors

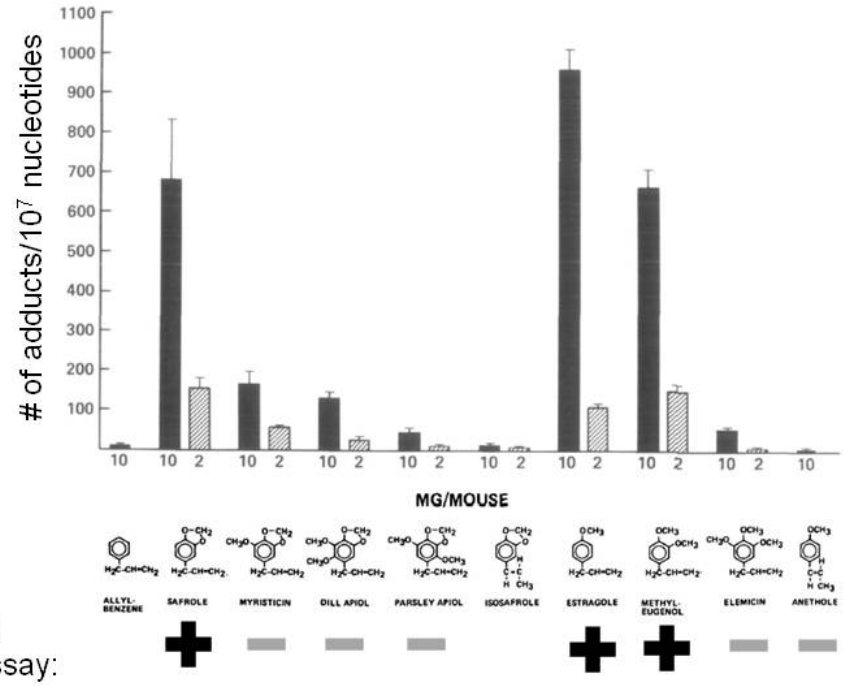
Review: Justification for class study

- Widespread, low dose exposure to an array of alkylanilines, a fraction of which are associated with cancer in the general population
- Likely similar metabolism and bioactivation
- Likely polymorphic metabolism / bioactivation that is genetically determined
- Likely similar carcinogenic mode of action – DNA adduction, genotoxicity

Proposed Approach

- Class study (14 alkylanilines) centered on mode of action
  - *In vivo* genotoxicity
    - DNA reactivity and mutagenicity/clastogenicity
  - Validate relationship between mode of action and carcinogenicity
  - Influence of genetic variation on genotoxicity

Why we think this approach will work- Alkenylbenzenes



New born mouse assay:

Randerath, et al., Carc., 1984

Key issue:
DNA reactivity

- Alkylanilines tend to be negative in Ames studies
- Alkylanilines tend to be positive in micronucleus studies
- The most sensitive metric of the genotoxic potential of the alkylanilines is DNA reactivity

Specific Aim 1a:
Conduct *in vivo* DNA reactivity studies

- Evaluations:
  - DNA adduct levels in multiple tissues
  - Hemoglobin adducts for purposes of scaling to human internal dose
- Doses:
  - 2 or more equimolar doses of all 14 alkylanilines
- Species:
  - mouse (C57BL/6)

Key Issue:
DNA reactivity → Permanent genetic change

- DNA reactivity does not always translate to mutagenicity / clastogenicity
- Establish relationship between DNA reactivity and permanent genetic change produced by the alkylanilines

Specific Aim 1b:
Conduct *in vivo* mutagenesis / clastogenesis studies

- Evaluations:
  - Performed in parallel with DNA adduct studies
  - *Pig-A* assay
  - Comet assay of select tissues
  - Erythrocyte micronuclei

Key Issue:
Genotoxicity → Carcinogenicity

- Need to establish the relationship between genotoxic potency and carcinogenic activity

Specific Aim 2:
Conduct transgenic mouse bioassay

- Perform short-term carcinogenicity bioassays
- *Xpc*$^{-/-}$/*p53*$^{+/-}$ knockout mice (C57BL/6 background)
  - sensitive to arylamine induced carcinogenic transformation in the urinary bladder and liver
- 4 or more of the 14 alkylanilines will be selected for study
  - 1 known carcinogen
  - 1 high potency genotoxin
  - 1 intermediate potency
  - 1 low potency

Key Issue:
Genetic susceptibility to alkylaniline genotoxicity

- Genetic variation in Phase I and Phase II enzymes confer differential carcinogenic risk associated with arylamine exposure
- Alkylanilines are anticipated to undergo polymorphic bioactivation

Specific Aim 3:
Conduct *in vitro* DNA adduct studies in hepatocytes from multiple inbred mouse strains

- Cultured hepatocytes from a subset of mice strains
  - Strains (including C57BL/6) will be selected based on genetic diversity at loci known to influence arylamine metabolism (N-acetyl transferase, CYP1A, glutathione -S-transferase)
- Evaluations:
  - DNA adduct formation
  - DNA damage using the Comet assay

Significance and Anticipated Outcome

- The proposed research program will determine, relative to known rodent carcinogens:
  - the genotoxic and mutagenic hazard of a collection of largely untested alkylanilines with widespread human exposure
  - the carcinogenic activity of a select subset of alkylanilines
  - the influence of genetic variation on alkylaniline genotoxicity
- Provide a framework for more efficient evaluation of the carcinogenic hazard associated with arylamine exposure
- Hb adducts will provide a metric of internal dosimetry for more accurate scaling of carcinogenic hazard to humans

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Questions?